Visual Input Regulates Circuit Configuration in Courtship Conditioning of *Drosophila melanogaster*

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Abstract

Courtship and courtship conditioning are behaviors that are regulated by multiple sensory inputs, including chemosensation and vision. Globally inhibiting CaMKII activity in *Drosophila* disrupts courtship plasticity while leaving visual and chemosensory perception intact. Light has been shown to modulate CaMKII-dependent memory formation in this paradigm and the circuitry for the nonvisual version of this behavior has been investigated. In this paradigm, volatile and tactile pheromones provide the primary driving force for courtship, and memory formation is dependent upon intact mushroom bodies and parts of the central complex. In the present study, we use the GAL4/UAS binary expression system to define areas of the brain that require CaMKII for modulation of courtship conditioning in the presence of visual, as well as chemosensory, information. Visual input suppressed the ability of mushroom body- and central complex-specific CaMKII inhibition to disrupt memory formation, indicating that the cellular circuitry underlying this behavior can be remodeled by changing the driving sensory modality. These findings suggest that the potential for plasticity in courtship behavior is distributed among multiple biochemically and anatomically distinct cellular circuits.

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Introduction

Courtship in *Drosophila* is a complex and robust behavior. Multiple sensory modalities (olfaction, vision, tactile sensation) combine to trigger a stereotyped output. Loss of any one of these sensory inputs by environmental, genetic, or surgical manipulation is insufficient to abolish courtship (for review, see Tompkins 1984). Loss of visual input results in a decrease in courtship level, but in *Drosophila melanogaster*, does not eliminate the behavior (Joiner and Griffith 1997). Loss of two inputs, vision and olfaction, also does not abolish courtship (Tompkins et al. 1980; Gailey et al. 1986), although loss of all three sensory modalities will virtually eliminate the behavior (Gailey et al. 1986). These studies suggest that there is redundancy in the stimuli that can elicit this behavior.

There is also overlap between the sensory inputs that stimulate courtship and modulate the behavior. Courtship is a plastic behavior. Males are able to modify their level of courtship based on prior experience (Siegel and Hall 1979). Exposure of a male to a previously mated female results in a suppression of subsequent courtship for 2–3 hr. This modulation is dependent on sensing and associating both stimulatory cues, which act as conditioned stimuli (CS) and aversive pheromonal cues, which act as unconditioned stimuli (US) that a mated female provides (Ackerman and Siegel 1986; Ferveur 1997).

Vision plays a role in this plasticity. Courtship conditioning will occur in the absence of visual input (Siegel and Hall 1979; Joiner and Griffith 1997), but vision is an important modulator of the formation of the associative memory of conditioning (Joiner and Griffith 1997). Flies with reduced central nervous system calcium/calmodulin-dependent protein kinase II (CaMKII) activity are defec-

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tive in both the ability to suppress courtship of a mated female during training and the ability to form a memory of this training (Griffith et al. 1993; Joiner and Griffith 1997). The defects in memory formation are seen only in the absence of normal visual input; visual signals during the conditioning period (but not during retrieval) can "rescue" the memory phenotype of flies expressing a CaMKII inhibitor peptide (Joiner and Griffith 1997). The importance of the visual input in this context is likely due to its courtship stimulatory properties rather than any role in the aversive nature of the mated female because the visually perceivable behavior of the female does not affect her aversiveness (Kamyshev et al. 1999). The cellular mechanism of this effect (whether it is due to multiple interacting sites of action of the inhibitor peptide or to an alternative signal transduction pathway) was not addressed.

These findings led to the formation of a simple model in which chemosensory and visual information interact at a CaMKII-dependent switch to regulate courtship behavior. Visual input acts in synergy with stimulatory pheromones as a positive, courtship promoting signal. Courtship suppression is produced by a negative signal, an aversive pheromone produced by the mated female. Suppression occurs when the strength of the negative pathway is greater than that of the positive pathway. Memory is encoded in a decrease in the efficacy of the positive cues to switch on courtship. Visual information is able to ameliorate the effects of CaMKII inhibition by greatly increasing the positive courtship signal and overcoming the change in strength of this connection.

Since this work on the interaction of visual input and CaMKII, anatomical regions that require CaMKII for courtship conditioning in the absence of visual input have been identified using GAL4 enhancer trap-driven expression of CaMKII inhibitor peptide (Joiner and Griffith 1999). This study strongly supported the idea that the response to the mated female during conditioning and the formation of associative memory were independent behavioral outputs of conditioning, because they were sensitive to inhibition of CaMKII activity in different areas of the brain. The conditioning response was found to be dependent on a subset of cells in the chemosensory antennal lobes and a subset of cells in the lateral protocerebrum. Associative memory formation was most sensitive to CaMKII inhibition in the mushroom bodies (primarily the γ lobes), the central complex (parts of the ellipsoid body and the fan-shaped body), and cells in the lateral protocerebrum. We defined an information flow circuit for courtship conditioning produced by chemosensory cues alone.

In this study we use GAL4 enhancer trap lines to address the question of how visual information affects the ability of CaMKII inhibitor peptide to disrupt the function of defined elements of the courtship conditioning circuit. Global inhibition of CaMKII in animals with normal vision has been shown to have no effect on memory formation (Joiner and Griffith 1997). In the present study, visual input abrogated the effects of CaMKII inhibition in the mushroom body and central complex on memory formation, rendering memory insensitive to CaMKII inhibition. Conversely, the behavior of the male toward the mated female during conditioning was, in general, more sensitive to inhibition of CaMKII when the male had visual input. Expression of inhibitor in the fan-shaped body, optic lobe, and a subset of the lateral protocerebrum effectively eliminated the normal response to the mated female only when the male received visual input. Mushroom body and chemosensory antennal glomerulus phenotypes during the conditioning period were unaffected by visual input. These results suggest that the functional circuitry underlying courtship conditioning can be dynamically rewired by sensory input.

Materials and Methods

DROSOPHILA STRAINS

Fly cultures were kept at 25°C with a 12 hr light/dark cycle on autoclaved cornmeal, yeast, sucrose, and agar food. The genetic background used for the behavior experiments was either from Canton-S or the line w^{1118}(isoCJ1) a *white*, Canton-S isogenic stock (Yin et al. 1994). In this text, *w* refers to this allele. Unless otherwise stated, genotypes are described in Lindsley and Zimm (1992). The GAL4/UAS system is described by Brand and Perrimon (1993). The UAS lines used in this study have the upstream activator sequences linked either to the ala-inhibitor peptide (UAS–ala; Joiner and Griffith 1997) or to a cDNA for the R3 isoform of *Drosophila* CaM kinase II (UAS–CaMKII; Koh et al. 1999). ala inhibitor is a synthetic peptide based on the sequence of the rat αCaMKII autoregulatory domain (Griffith et al. 1993). F_1 males that were tested in the learning assay resulted from crossing virgin females, homozygous for the GAL4 insert, to

males homozygous for the UAS–ala insert, both the UAS–ala and UAS–CaMKII inserts, or, for controls, w^{1118}(isoCJ1).

BEHAVIOR ASSAYS

COURTSHIP CONDITIONING ASSAY

Singly housed, 5-day-old test males were placed with 4-day-old females, mated the previous day, in single-pair-mating chambers (8 mm diam. × 3 mm high) for 1 hr. The first and last 10-min periods of this conditioning period were videotaped. Two to 5 min after conditioning, the males were paired in a clean mating chamber with anesthetized virgin females that had been collected that day. The pairs were videotaped for the 10-min test period. As a control, sham tests are done in which the males are kept alone in the mating chamber for the first hour, then paired with anesthetized virgin females for the 10-min test period. For each of the 10-min periods, a courtship index (CI) was measured for each male tested. The fraction of time a male spent courting in a 10-min interval constitutes the CI. The response to the mated female is calculated by dividing the final CI (CI_{final}) by the CI of the initial 10-min period ($CI_{initial}$). $CI_{initial}$ did not vary significantly from that of wild type (Dunnett's test, $\alpha = 0.05$; $\alpha = 0.03$ for MJ63/+). Because males used for the test period are not the same as those in the sham test, memory is measured by dividing the test CI (CI_{test}) with the average (mean) sham test CI (mCI_{sham}). CI_{sham} for all lines was indistinguishable statistically from wild type (Dunnett's test, $\alpha = 0.05$). Females used in this assay were collected from a *shi^{ts}/Y/C(1)DX*, *y w f* stock. When kept at 29°C, only *C(1)DX*, *y w f* females emerge from this stock. Experiments were performed at 25°C and 75% humidity. Unless otherwise indicated, all behavior experiments were done under normal room light. In experiments done in red light, two lamps with 25-W red photographic light bulbs were placed 20 cm from the mating wheel. $N \geq 20$ for all genotypes. UAS–ala/+ flies were shown previously to behave normally in the courtship conditioning assay (Joiner and Griffith 1997).

LOCOMOTOR ACTIVITY ASSAY

Spontaneous locomotor activity was measured by counting the number of times a fly crosses a line drawn across an 8-mm-diameter circular chamber in a 4-min period.

STATISTICS

Data for the courtship conditioning assay were analyzed with Wilcoxon's signed-rank test using Statview software version 4.5 for the Macintosh. Data are presented in the figures as means with levels of significance indicated by P values: (*) < 0.05; (**) < 0.005; (***) < 0.0005. In cases where comparisons for groups were done, the Kruskal–Wallis rank sums test was used. For multiple pairwise comparisons, levels of significance were calculated using an experiment-wise correction factor, $\alpha = 1 - (0.95)1/k$, where k is the number of pairwise comparisons and α is the new significance level (Sokal and Rohlf 1995; Kane et al. 1997).

Results

In a previous study we used GAL4-directed expression of a CaMKII inhibitor peptide to map the areas of the brain that require this signal transduction pathway for conditioning in the absence of visual input (Joiner and Griffith 1999). The GAL4 system allows for tissue-specific expression of transgenes (Brand and Dormand 1995). Each enhancer trap line expresses GAL4 in a particular pattern depending on the local environment of the *GAL4* gene. We have characterized lines that express *GAL4* in adult brain areas that are important for courtship conditioning, namely mushroom bodies, central complex, antennal glomerulus, and lateral protocerebrum (Joiner and Griffith 1999). A representative subset of these lines was used to drive expression of ala peptide, a specific inhibitor of CaMKII (Griffith et al. 1993), in order to investigate the effects of vision on the major components of the circuit.

Males were trained for 1 hr with a mated female in an 8-mm-diameter mating chamber. Training was carried out under normal room lights to allow males to receive visual input as well as chemosensory cues. The behavior of the male toward the mated female during training was assessed by measuring the amount of courtship performed during the first and last 10 min of the training period. The level of courtship is expressed as CI. A CI of 1 indicates that the male courted for the entire 10-min observation period. For a normal male, the

ratio of the final and initial 10-min observations ($CI_{final}/CI_{initial}$) during training is usually ~0.5 (Joiner and Griffith 1997, 1999). $CI_{initial}$ values for all lines were indistinguishable statistically from that of wild type (data not shown). After training, the male was transferred within 2–5 min to a clean chamber and presented with a virgin female. Virgins produce stimulatory pheromones (CS), but not aversive pheromones (US). Associative memory formation was assessed by measuring CI_{test} during the first 10 min of exposure to the virgin female. For normal males, CI_{test} will be significantly lower than CI_{sham}. The ratio of CI_{test} to the mCI_{sham} for that genotype is typically 0.5 for wild-type males (Joiner and Griffith 1999). For all genotypes, the CI_{sham} was indistinguishable statistically from that of wild type (data not shown). For both the training and memory phases of the assay, GAL4/+; UAS–ala/+ males are compared to GAL4/+ control males, which express no inhibitor but contain the same GAL4 transgene. UAS–ala/+ control animals behave indistinguishably from wild-type animals (Joiner and Griffith 1997).

Data for the courtship response during training with visual input are shown in Figure 1. Normal male flies will show a decrement in courtship during training with a $CI_{final}/CI_{initial}$ ratio of ~0.5. Of the three lines (29BD, 30Y, 201Y) with predominant mushroom body expression that were tested, only expression of inhibitor under control of 201Y produced an abnormally high ratio during the training period. This line was also abnormal when tested in the absence of visual input (Table 1). Expression of inhibitor in the fan-shaped body of the central complex (OK348) also produced a behavioral phenotype, but in this case, the phenotype was seen only with visual input (Table 1). Expression of inhibitor in the chemosensory antennal lobes (MJ94) produced a behavioral phenotype both in the presence and the absence of visual input. 40B, which has weak expression in a number of areas including the optic lobes, is abnormal only in the presence of visual input. Lines with predominant expression in the lateral protocerebrum show a complex response to visual stimulation, suggesting that the lateral protocerebrum is not a homogeneous structure. Lateral protocerebrum expression can produce both vision-dependent (MJ162a, MJ146, MJ63) and vision-independent phenotypes (201Y).

Data for the memory assay in white light are shown in Figure 2. In no case was expression of the inhibitory peptide sufficient to disrupt memory formation. This is in contrast to the results for the same lines in the absence of visual input (Table 2). In the absence of vision, expression of the peptide in mushroom bodies, central complex, and a subset of the lateral protocerebrum blocked formation of memory (Joiner and Griffith 1999).

The changes in courtship activity were not the result of changes in locomotor activity. The progeny of GAL4 lines crossed to UAS–ala flies were tested for locomotion in the courtship assay chambers. None of the lines showed a significant difference in locomotion in the line-crossing test when compared pairwise ($P > 0.05$, Tukey–Kramer Test; Table 3). Previous tests of these genotypes in dim red light also failed to show any affect of CaMKII inhibition on locomotion, even after flies spent an hour in the conditioning chamber (Joiner and Griffith 1997), ruling out fatigue as a cause of the courtship phenotypes.

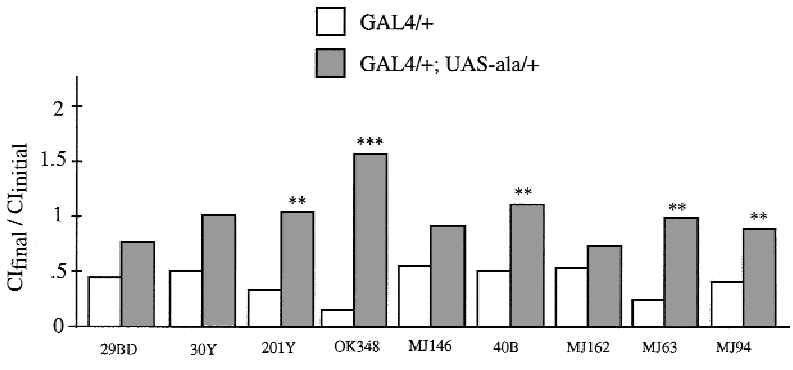

Figure 1: Inhibition of CaMKII in specific brain regions alters the response to the mated female during conditioning. Males heterozygous for the GAL4 lines indicated were trained by exposure to a female for 1 hr. A CI is measured for the first and last 10 min of the training period. Courtship decrement during training is shown by the ratio of $CI_{final}/CI_{initial}$, which for a wild-type male is ~0.5 (Joiner and Griffith 1997). All manipulations were performed at 25°C, 75% humidity in normal room light. Statistical significance was assessed by Wilcoxon's signed-rank test. Data from GAL4/+;UAS–ala/+ males (gray bars) were compared to data from control males of the genotype GAL4/+ (white bars) for each line. Bars marked with an asterisk (*) are significantly different from the genotype control with level of significance as indicated: (*) $P < 0.05$; (**) $P < 0.005$; and (***) $P < 0.0005$. $n \geq 20$ for each genotype.

Table 1: *Effects of light on the response to mated females*

Line	Light: behavior in white	Light: behavior in red	Expression pattern
29BD	normal	normal	**MB (core of α and β, all of γ)** LP, OL, PI, and AL (weak)
30Y	normal	normal	**MB (strong in α, β and γ),** LP and PI (weak)
201Y	abnormal	abnormal	**MB (core of α and β, all of γ),** LP (two pairs of cells), PI
OK348	abnormal	normal	**FSB**
40B	abnormal	normal	MB (weak β), OL, AL (ventral)
MJ162a	normal	abnormal	**LP (diffuse),** MB (weak), AL (ventral), palps, PI
MJ146	normal	abnormal	**PI, LP (lateral and ventral),** MB (weak, core of α and β)
MJ63	abnormal	normal	**LP (medial), neural tracts traversing the central brain,** PI
MJ94	abnormal	abnormal	**AL (chemosensory)** palps and MB (very weak β)

Behavioral phenotypes during training are compared for GAL4-driven expression of ala peptide in the presence (behavior observed under white lights; Fig. 1) or absence (behavior observed under red lights; Joiner and Griffith 1999) of visual input. "Normal" indicates $P > 0.05$ for the comparison between the $CI_{final}/CI_{initial}$ ratios of GAL4/+, UAS–ala/+, and GAL4/+ genotypes. "Abnormal" indicates $P < 0.05$ for this comparison. The expression patterns for each GAL4 line are summarized in the last column. The primary area of expression is indicated in boldface type, with less prominent expression areas indicated in normal type. (MB) Mushroom bodies; (FSB) fan-shaped body; (LP) lateral protocerebrum; (PI) pars intercerebralis; (AL) antennal lobes; (OL) optic lobes. Anatomical data are summarized from Joiner and Griffith (1999).

The specificity of the effects of this inhibitor peptide and its interaction with visual input in this behavior were tested by asking if addition of exogenous CaMKII activity could rescue the light-dependent and light-independent behavioral phenotypes. For this experiment, a globally expressing GAL4 line, MJ85b (Joiner and Griffith 1997), was used. Expression of ala peptide under control of this line has been shown to significantly disrupt the response to the mated female during training (both with and without visual input), but to disrupt memory formation only in the absence of visual input (Fig. 3; Joiner and Griffith 1997). In all of these cases, addition of exogenous CaMKII by a UAS transgene was able to improve behavior significantly. In the case of memory formation in red light, rescue is complete; the CI_{test}/mCI_{sham} ratio of MJ85b;UAS–ala/+;UAS–CaMKII/+ is reduced to the same level as the control, MJ85b ($P < 0.0005$ comparing MJ85b;UAS–ala/+ to MJ85b;UAS–ala/+;UAS–CaMKII/+ using Wilcoxon's signed rank test). In the case of the response to the mated female during training, rescue is only partial; $CI_{final}/CI_{initial}$ ratios are reduced significantly in

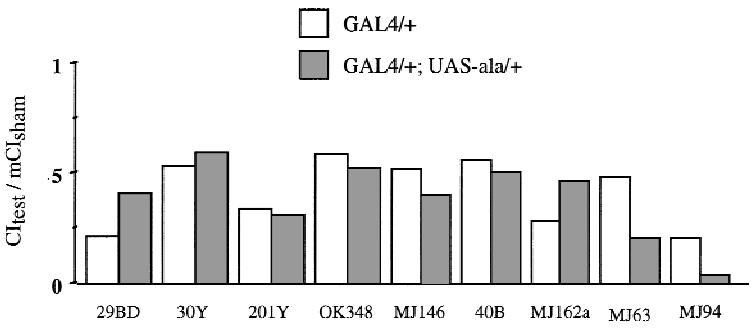

Figure 2: Inhibition of CaMKII in specific brain areas does not alter associative memory formation. Males heterozygous for the GAL4 lines indicated were trained by exposure to a female for 1 hr and placed with an anesthetized virgin female. For sham training, flies of the same genotype were manipulated identically, except that no female was present in the chamber during training. Memory was tested by measuring the CI for the initial 10 min of exposure to the anesthetized virgin female after training with a mated female (CI_{test}) or after sham training (CI_{sham}). Data are expressed as the ratio of CI_{test}/mean CI_{sham}, which, for a wild type male, is ~0.5 (Joiner and Griffith 1997). All manipulations were performed at 25°C, 75% humidity in normal room light. Statistical significance was assessed by Wilcoxon's signed-rank test. Data from GAL4/+;UAS–ala/+ males (gray bars) were compared to data from control males of the genotype GAL4/+ (white bars) for each line. Bars marked with an asterisk (*) are significantly different from the genotype control with level of significance as indicated: (*) $P < 0.05$, (**) $P < 0.005$, and (***) $P < 0.0005$. $n \geq 20$ for each genotype.

Table 2: *Effects of light on memory*

	Light		
Line	behavior in white	behavior in red	Expression pattern
29BD	normal	abnormal	**MB (core of α and β, all of γ),** LP, OL, PI, and AL (weak)
30Y	normal	abnormal	**MB (strong in α, β and γ),** LP and PI (weak)
201Y	normal	abnormal	**MB (core of α and β, all of γ),** LP (two pairs of cells), PI
OK348	normal	abnormal	**FSB**
40B	normal	normal	MB (weak β), OL, AL (ventral)
MJ162a	normal	normal	**LP (diffuse)** MB (weak), AL (ventral), palps, PI
MJ146	normal	normal	**PI, LP (lateral and ventral),** MB (weak, core of α and β)
MJ63	normal	abnormal	**LP (medial), neural tracts traversing the central brain,** PI
MJ94	normal	normal	**AL (chemosensory),** palps and MB (very weak β)

Memory phenotypes after training are compared for GAL4-driven expression of ala peptide in the presence (behavior observed under white lights; Fig. 2) or absence (behavior observed under red lights; Joiner and Griffith 1999) of visual input. "Normal" indicates $P > 0.05$ for the comparison between the CI_{test}/mCI_{sham} ratios of GAL4/+; UAS–ala/+ and GAL4/+ genotypes. "Abnormal" indicates $P < 0.05$ for this comparison. See Table 1 for abbreviations and explanations. Anatomical data are summarized from Joiner and Griffith (1999).

comparison to MJ85b;UAS–ala/+, but they are not as low as control ($P < 0.05$ using Wilcoxon's signed rank test for both light conditions). Memory formation in white light was unaffected by any manipulation ($P > 0.5$, group comparison done using Kruskal–Wallis rank sums test). The effects of expression of CaMKII were specific; expression of green fluorescent protein (GFP) via a UAS–GFP construct did not rescue the UAS–ala phenotype or modulate courtship levels (data not shown). These results indicate that the actions of the ala peptide are mediated primarily by CaMKII.

Table 3: *Locomotor activity*

Genotype	Line crossing
29BD/+; UAS–ala/+	138 ± 5
30Y/+; UAS–ala/+	116 ± 5
201Y; UAS–ala/+	113 ± 5
OK348/+; UAS–ala/+	121 ± 9
40B/+; UAS–ala/+	115 ± 10
MJ162a; UAS–ala/+	112 ± 7
MJ146; UAS–ala/+	128 ± 5
MJ63; UAS–ala/+	117 ± 9
MJ94; UAS–ala/+	112 ± 4

Locomotor activity was measured by counting spontaneous line crossings in the courtship conditioning chamber for 4 min. Assay was performed in normal room light; $n = 10$ for all genotypes. Data are presented as mean ± S.E.M. No significant differences were seen ($P > 0.05$) using a Tukey-Kramer comparison of all pairs. Data for MJ85b; UAS–ala/+ and Canton S control have been reported previously and were not significantly different (Joiner and Griffith 1997).

Discussion

COURTSHIP CONDITIONING IS A MULTIMODAL BEHAVIOR

Courtship can be stimulated and modulated by multiple types of sensory input. It has been known for many years that vision is able to modulate basal courtship levels and courtship success (Geer and Green 1962; Grossfield 1966; Connolly et al. 1969; DeJianne et al. 1981; Tompkins et al. 1982; Markow 1987; Chatterjee and Singh 1988; Stocker and Gendre 1989; Joiner and Griffith 1997). Vision appears to act as a positive stimulus to courtship. Courtship in the absence of visual input is normal in its details (steps and sequence) but is reduced in intensity, as reflected in lower CI values. Courtship conditioning, suppression of subsequent courtship after exposure to a mated female, is observed both with and without visual input (Siegel and Hall 1979; Joiner and Griffith 1997). This plasticity is thought to be based on an association of positive and negative cues that the male receives from mated females. The negative cue is believed to be an aversive pheromone that is synthesized only by mated females in response to courtship (Tompkins and Hall 1981; Tompkins et al. 1983; Gailey and Siegel 1989). The positive courtship cues include

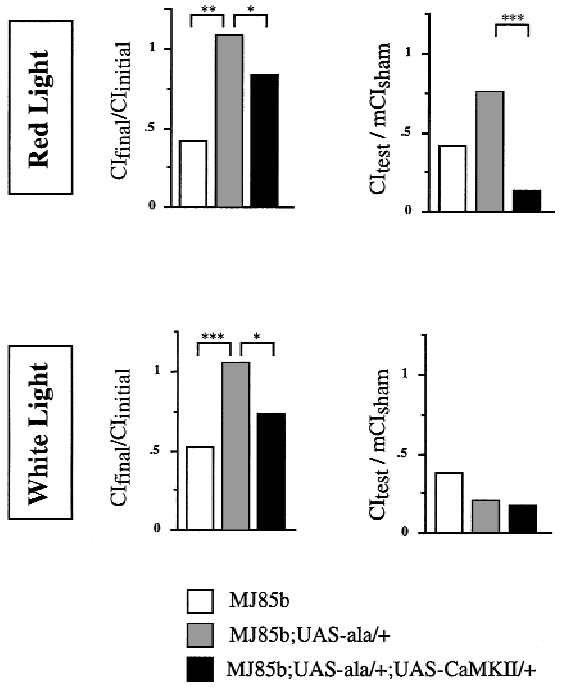

Figure 3: Overexpression of CaMKII rescues both the response to the mated female and associative memory formation. CaMKII inhibitor was driven using MJ85b, a GAL4 line that expresses throughout the brain. The response to the mated female during conditioning and memory formation were measured as described in Materials and Methods and in Figures 1 and 2. All manipulations were performed at 25°C, 75% humidity in either dim red light (*top* two panels) or normal room light (*bottom* two panels). Statistical significance was assessed by Wilcoxon's signed-rank test. Data from MJ85b;UAS–ala/+ males (gray bars) were compared with data from MJ85b;UAS–ala/+; UAS–CaMKII males (black bars) and data from MJ85b controls (white bars). Bars marked with an asterisk (*) are significantly different from the genotype control with level of significance as indicated: (*) $P < 0.05$; (**) $P < 0.005$; and (***) $P < 0.0005$. $n \geq 20$ for each genotype.

both volatile and tactile pheromones and visual input (Gailey et al. 1986). Visual input is not necessary for plasticity, but acts to enhance the drive to court. Thus, depending on how the assay is conducted (in normal room light or in dim red light which is above the wavelength of perception for flies) courtship conditioning can be a purely chemosensation-driven behavior or it can be a multimodal (visual and chemosensory) behavior.

CaMKII has been shown to be required for the modification of courtship during training in the multimodal courtship conditioning paradigm. In the absence of visual input, when the behavior is driven solely by chemosensory cues, CaMKII is required for both the decrement of courtship during conditioning and the formation of associative memory of conditioning (Griffith et al. 1993, 1994; Joiner and Griffith 1997, 1999). The two outputs of this conditioning, the response during training and the subsequent memory, are independent products of the conditioning based on the fact that they are dependent on different brain structures (Joiner and Griffith 1999) and can be dissociated by biochemical manipulations (Joiner and Griffith 1997; Kane et al. 1997).

THE CIRCUITRY USED FOR ASSOCIATIVE MEMORY FORMATION IS SENSORY MODALITY-DEPENDENT

The circuitry underlying the CaMKII-dependent formation of associative memory has been investigated using cell-specific expression of a CaMKII inhibitor (Joiner and Griffith 1999). Memory formation in the absence of visual input was found to be dependent on CaMKII activity in the mushroom bodies and parts of the central complex (fan-shaped body and a subset of the ellipsoid body). In this study we present evidence that the circuitry utilized for memory formation in the presence of visual input is different. Using the same GAL4 lines that were used in the previous mapping study, we find that memory formation is insensitive to CaMKII inhibition when there is visual input driving courtship. We postulate that different sets of cells can be used in memory formation depending on the sensory inputs that are driving the behavior.

This result could be interpreted in two ways. The first interpretation is that the mushroom bodies and central complex are not used for memory formation when visual information is present; the circuit has been reconfigured and different neural pathways are used. A second possibility is that these central brain neurons are still used, but the biochemical pathways mediating plasticity are altered, that is, CaMKII itself is no longer required. This second possibility seems less likely because if chemosensory input stimulates CaMKII, this input is still present in the light and would presumably still activate this pathway. The first possibility is also supported by the results of other researchers who have examined the role of mushroom bodies in plastic behaviors. In an examination of a wide variety of behavioral paradigms, Heisenberg's group found that behaviors that were primarily driven by visual, tactile, or motor inputs were insensitive to ablation of mushroom bodies by hy-

droxyurea, while olfactory-based learning was disrupted (Wolf et al. 1998). Memory formation of courtship conditioning has also been shown to be unimpaired in hydroxyurea treated animals (K. Siwicki, pers. comm.). It is important to note that a role for mushroom bodies in long-term memory or some post-learning consolidation is not ruled out based on these studies because they all involved short term memory or involved immediate recall in the case of courtship conditioning. In any case, these studies present a picture of the mushroom bodies as an important integration site only for olfactory behaviors. It is clear that the mushroom bodies receive information from visual pathways (Barth and Heisenberg 1997) but this information is apparently not used for modulation of courtship behavior.

An additional fallout of these experiments and experiments in which CaMKII is inhibited globally (Joiner and Griffith 1997) is that memory formed using visual information is biochemically distinct from that formed when only chemosensory input is used. The failure of global inhibition of CaMKII to block memory formation in the multimodal assay argues that, in this paradigm, plasticity is dependent on an alternative signal transduction pathway. Experiments done with transgenic flies expressing an inhibitor of protein kinase C (Kane et al. 1997), in which memory formation was unaffected, make it unlikely that this kinase is involved. The failure of learning mutants involved in cAMP-dependent signal transduction to learn in this assay (Gailey et al. 1984; Ackerman and Siegel 1986) makes this pathway an attractive candidate for the mediator of memory formation in the multimodal assay. Where this alternative signal transduction pathway is functioning (e.g. in the mushroom bodies or elsewhere) is unknown. It is possible that the parallel "circuit" we have uncovered is a biochemical pathway within the mushroom body or central complex as opposed to being in a separate set of cells. The ablation experiments of Heisenberg and Siwicki would argue for a cellular pathway with regard to the mushroom body, but until the other signal transduction system is identified and mapped, the issue is open to debate.

THE CIRCUITRY USED FOR MODULATION OF COURTSHIP DURING TRAINING IS SENSORY MODALITY DEPENDENT

The situation for the other behavioral output of courtship conditioning, the decrement of courtship during the training period, is more complex. In the previous study, in the absence of visual input, this behavior was found to be most sensitive to inhibition of CaMKII in antennal lobes and parts of the lateral protocerebrum. These areas are relatively early in the circuit. The antennal lobes get direct chemosensory input and have separate projections to areas of the lateral protocerebrum and to the mushroom bodies (Stocker et al. 1990, 1997). Cells in the lateral protocerebrum project to both mushroom bodies and central complex, areas critical for memory formation in this assay.

In general, there is a greater sensitivity to CaMKII inhibition in the presence of visual input, with a number of lines that showed no behavioral phenotype in red light becoming abnormal when the male was able to see. These include lines that express in the fan-shaped body of the central complex (OK348), the lateral protocerebrum (MJ63), and a line with low expression in a wide variety of areas (40B). There are also two lateral protocerebral lines that show a suppression of phenotype with visual input (MJ146 and MJ162a). This complicated pattern points to an involvement of the lateral protocerebrum in the stimulation of courtship by visual input. This is not surprising given the visual projections from the lobula plate to the lateral protocerebrum in insects (Strausfeld 1976). The lobula plate also projects to the central complex, perhaps explaining the vision-dependent phenotype of OK348. In the male fleshfly *Sarcophaga bullata*, this projection includes neurons that are gender-specific and aid in tracking females (Gilbert and Strausfeld 1991; Douglass and Strausfeld 1998). This gender-specific projection has not been documented in *Drosophila*. Visual information may also interact with chemosensory information in the lateral protocerebrum (Li and Strausfeld 1999).

CIRCUIT RECONFIGURATION ALLOWS BEHAVIORAL FLEXIBILITY

What benefit do modality-dependent circuits confer to the animal? Reproductive behavior is critical to species survival. The ability of a wide range of sensory inputs to stimulate this behavior underscores its importance. One way to design such a system would be to have a single set of neurons integrating primary inputs and gating motor output (Fig. 4A). This would achieve the goal of allowing multiple stimuli to trigger and modulate behavior. An alternative way of organizing the sys-

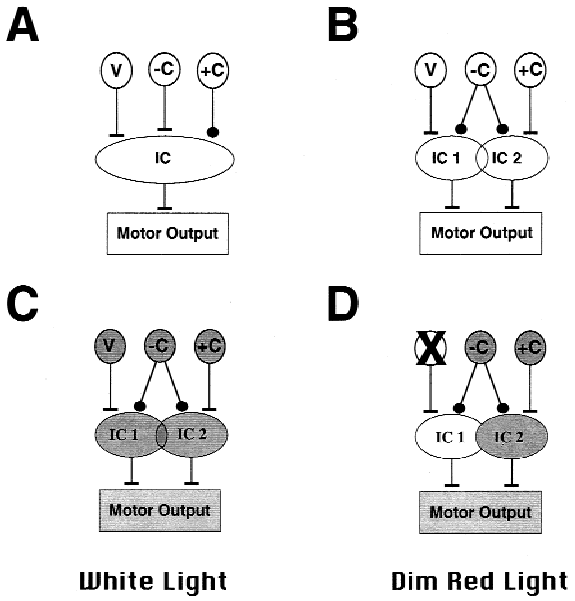

Figure 4: Multimodal behavioral circuits. Stimulatory connections between neural centers are indicated by (a T-bar) and inhibitory connections by (a solid circle). Stimulatory sensory inputs include vision (V) and positive chemosensory cues (+C). Inhibitory sensory inputs include aversive chemosensory cues (–C). Sensory inputs are integrated at one or more ICs to modulate behavior via the motor center. (*A*,*B*) Possible configurations of circuits that allow multiple sensory inputs to stimulate and modulate a behavior. In *A*, redundant sensory inputs feed into a single integration center that controls motor output. In *B*, redundant sensory inputs feed into IC 1 and IC 2, which can act independently. ICs are drawn to contain a subset of shared elements, although this need not be the case (see text). (*C*) A circuit with multiple integration centers operating under conditions where all sensory pathways are operative. In this case, both IC 1 and IC 2 are operative and redundant, that is, inactivation of one IC will not prevent modulation of behavior. Shading indicates neural centers that are active. (*D*) The circuit working in the absence of visual input. In this case, the only functional integration center is IC 2. Inactivation of this IC will prevent modulation of behavior.

tem is to design parallel circuits that respond to single or specific combinations of sensory inputs (Fig. 4B). This would maximize the redundancy of the system, an advantage for a critical behavior.

This second type of model is suggested by our data for the circuitry underlying the two behavioral outputs of courtship conditioning. Figure 4, C and D, shows how the activation of different sensory pathways reconfigures the circuit. In Figure 4C, all pathways are active and learning can occur at both 1 integration centers 1 and 2 (IC1, IC2). Inactivation of one integration center does not disrupt learning. In Figure 4D, when visual input is removed, only one integration center is used. In this case, inactivation of IC 2 will disrupt modulation. In the case of associative memory formation in courtship conditioning, the mushroom bodies and central complex would be analogous to IC 2.

The circuits may or may not interact or use common elements. Our data for the response to the mated female during conditioning suggest that there are common elements shared by visual and nonvisual integration centers such as antennal lobes and parts of the lateral protocerebrum. This would be analogous to the overlap between IC 1 and IC 2. In contrast, memory formation centers appear to be nonoverlapping as even global inhibition of CaMKII fails to prevent memory formation in the presence of visual input. IC 2 would represent CaMKII-dependent elements, whereas IC 1 relies on a different signal transduction pathway and does not share elements with IC 2.

The major advantage to the organism in such circuitry rests on the fact that not only are there multiple types of stimuli that can elicit a behavior, but there are also several sites for its modification. The existence of multiple integration centers ensures that even if one circuit is damaged, the animal can still learn. This type of distributed system allows adaptation after injury (Rauschecker 1995). Interplay between sites of plasticity could also be important in organization of hierarchical relationships between stimuli.

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References

Ackerman, S.L. and R.W. Siegel. 1986. Chemically reinforced conditioned courtship in *Drosophila*: Responses to wild-type and the *dunce, amnesiac* and *don giovanni* mutants. *J. Neurogenet.* **3:** 111–123.

Barth, M. and M. Heisenberg. 1997. Vision affects mushroom bodies and central complex in *Drosophila melanogaster. Learn. & Mem.* **4:** 219–229.

Brand, A.H. and E.L. Dormand. 1995. The GAL4 system as a tool for unraveling the mysteries of the *Drosophila* nervous system. *Curr. Opin. Neurobiol.* **5:** 572–578.

Brand, A.H. and N. Perrimon. 1993. Targeted gene

expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118:** 401–415.

Chatterjee, S. and B.N. Singh. 1988. Effect of light and dark on mating behavior of red eye and white eye *Drosophila ananassae. Ind. J. Exp. Biol.* **26:** 611–614.

Connolly, K., B. Burnet, and D. Sewell. 1969. Selective mating and visual pigmentation: An analysis of the visual component in the courtship behavior of *Drosophila melanogaster. Evolution* **23:** 548–559.

DeJianne, D., A. Pruzan-Hotchkiss, and J. Grossfeld. 1981. Light dependent mating inhibition in the white-eyed mutant of *Drosophila pseudoobscura. Experientia* **37:** 465–466.

Douglass, J.K. and N.J. Strausfeld. 1998. Functionally and anatomically segregated visual pathways in the lobula complex of a calliphorid fly. *J. Comp. Neurol.* **396:** 84–104.

Ferveur, J.-F. 1997. The pheromonal role of cuticular hydrocarbons in *Drosophila melanogaster. BioEssays* **19:** 353–358.

Gailey, D.A., F.R. Jackson, and R.W. Siegel. 1984. Conditioning mutations in *Drosophila melanogaster* affect an experience-dependent behavioral modification in courting males. *Genetics* **106:** 613–623.

Gailey, D.A., T.C. Lacaillade, and J.C. Hall. 1986. Chemosensory elements of courtship in normal and mutant, olfaction-deficient *Drosophila melanogaster. Behav. Genet.* **16:** 375–405.

Gailey, D.A. and R.W. Siegel. 1989. A mutant strain in *Drosophila melanogaster* that is defective in courtship behavioral cues. *Anim. Behav.* **38:** 163–169.

Geer, B.W. and M.M. Green. 1962. Genotype, phenotype and mating behavior of *Drosophila melanogaster. Am. Nat.* **96:** 175–181.

Gilbert, C. and N.J. Strausfeld. 1991. The functional organization of male-specific visual neurons in flies. *J. Comp. Physiol.* **169:** 395–411.

Griffith, L.C., L.M. Verselis, K.M. Aitken, C.P. Kyriacou, and R.J. Greenspan. 1993. Inhibition of calcium/calmodulin-dependent protein kinase in *Drosophila* disrupts behavioral plasticity. *Neuron* **10:** 501–509.

Griffith, L.C., J. Wang, Y. Zhong, C.F. Wu, and R.J. Greenspan. 1994. Calcium/calmodulin-dependent protein kinase II and potassium channel subunit Eag similarly affect plasticity in *Drosophila. Proc. Natl. Acad. Sci.* **91:** 10044–10048.

Grossfield, J. 1966. The influence of light on the mating behavior of *Drosophila. Univ. Tex. Publ.* **6615:** 147–176.

Joiner, M.A. and L.C. Griffith. 1997. CaM kinase II and visual input modulate memory formation in the neuronal circuit controlling courtship conditioning. *J. Neurosci.* **17:** 9384–9391.

———. 1999. Mapping of the anatomical circuit of CaM kinase-dependent courtship conditioning in *Drosophila. Learn. & Mem.* **6:** 177–192.

Kamyshev, N.G., K.G. Iliadi, and J.V. Bragina. 1999. *Drosophila* conditioned courtship: Two ways of testing memory. *Learn. & Mem.* **6:** 1–20.

Kane, N.S., A. Robichon, J.A. Dickinson, and R.J. Greenspan. 1997. Learning without performance in PKC-deficient *Drosophila. Neuron* **18:** 307–314.

Koh, Y.H., E. Popova, U. Thomas, L.C. Griffith, and V. Budnik. 1999. Regulation of DLG localization at synapses by CaMKII-dependent phosphorylation. *Cell* **98:** 353–363.

Li, Y. and N.J. Strausfeld. 1999. Multimodal efferent and recurrent neurons in the medial lobes of cockroach mushroom bodies. *J. Comp. Neurol.* **409:** 647–663.

Lindsley, D.L. and G.G. Zimm. 1992. *The genome* of Drosophila melanogaster. Academic Press, San Diego, CA.

Markow, T.A. 1987. Behavioral and sensory basis of courtship success in *Drosophila melanogaster. Proc. Natl. Acad. Sci.* **84:** 6200–6204.

Rauschecker, J.P. 1995. Compensatory plasticity and sensory substitution in the cerebral cortex. *Trends Neurosci* **18:** 36–43.

Siegel, R.W. and J.C. Hall. 1979. Conditioned responses in courtship behavior of normal and mutant *Drosophila. Proc. Natl. Acad. Sci.* **76:** 565–578.

Sokol, R.R. and H.R. Rohlf. 1995. *Biometry.* W.H. Freeman & Co., New York, NY.

Stocker, R.F. and N. Gendre. 1989. Courtship behavior of *Drosophila* genetically or surgically deprived of basiconic sensilla. *Behav. Genet.* **19:** 371–385.

Stocker, R.F., M.C. Lienhard, A. Borst, and K.F. Fischbach. 1990. Neuronal architecture of the antennal lobe in *Drosophila melanogaster. Cell Tissue Res.* **262:** 9–34.

Stocker, R.F., G. Heimbeck, N. Gendre, and J.S. de Belle. 1997. Neuroblast ablation in *Drosophila* P[GAL4] lines reveals origins of olfactory interneurons. *J. Neurobiol.* **32:** 443–456.

Strausfeld, N. J. 1976. Mosaic organizations, layers, and visual pathways in the insect brain. In *Neural principles in vision* (ed. F. Zettler and R. Weiler), pp. 245–279. Springer, Berlin, Germany.

Tompkins, L. 1984. Genetic analysis of sex appeal in *Drosophila. Behav. Genet.* **14:** 411–440.

Tompkins, L. and J.C. Hall. 1981. The different effects on

courtship of volatile compounds from mated and virgin *Drosophila* females. *J. Insect Physiol.* **27:** 17–21.

Tompkins, L., J.C. Hall, and L.M. Hall. 1980. Courtship-stimulating volatile compounds from normal and mutant *Drosophila. J. Insect Physiol.* **26:** 689–697.

Tompkins, L., A.C. Gross, J.C. Hall, D.A. Gailey, and R.W. Siegel. 1982. The role of female movement in the sexual behavior of *Drosophila melanogaster. Behav. Genet.* **12:** 295–307.

Tompkins, L., R.W. Siegel, D.A. Gailey, and J.C. Hall. 1983. Conditioned courtship in *Drosophila* and its mediation by chemical cues. *Genetics* **13:** 565–578.

Wolf, R., T. Wittig, L. Liu, G. Wustmann, D. Eyding, and M. Heisenberg. 1998. *Drosophila* mushroom bodies are dispensable for visual, tactile, and motor learning. *Learn. & Mem.* **5:** 166–178.

Yin, J.C.P., J.S. Wallach, M. DelVecchio, E.L. Wilde, H. Zhou, W.G. Quinn, and T. Tully. 1994. Induction of a dominant-negative CREB transgene specifically blocks long-term memory in *Drosophila. Cell* **79:** 49–58.

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